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Vesicles from simple synthetic amphiphiles as membrane mimetic systems. Fusogenic and polymorphic behavior.

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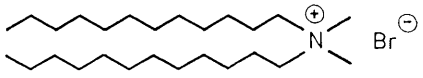
SUMMARY

This thesis describes the first detailed study of the fusogenic behavior of vesicles prepared from simple synthetic amphiphiles. Fusion, an important and frequently occurring process in nature, involves the close apposition of two membranes that become transiently unstable and subsequently merge. It occurs during processes such as virus infection, cell division, as well as endo- and exocytosis. Because biological membranes have a complex composition, *i.e.* they consist of a variety of lipids and proteins, the mechanism of fusion has been studied frequently using simple model systems such as phospholipid vesicles (liposomes). Like biological membranes, vesicles formed from both natural and synthetic phospholipids consist of a bilayer of lipids that encloses an aqueous compartment.

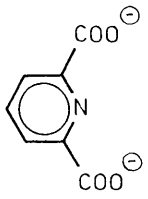
The structural variety of phospholipids and their amenability to chemical modifications is rather limited. Yet, the structure of the bilayer-forming molecule is of crucial importance, as it determines the stability of the bilayer and the properties of the bilayer-water interface. These parameters, in turn, play an essential role in membrane fusion. It is conceivable then, that studies concerning the mechanism of intermembrane interactions (including fusion) would be greatly facilitated by the synthetic availability of lipid-like molecules which have a structural variability greater than that of phospholipids. In principle, synthetic amphiphiles provide such a source of lipid-like molecules. Indeed, in the present study it is shown that vesicles prepared from synthetic amphiphiles can mimic such a specialized membrane function as fusion. A detailed investigation of the mechanism of fusion in these systems is described. During the course of this study, it appeared that synthetic amphiphiles can also display polymorphic properties, *i.e.* they are capable of undergoing a transformation from the bilayer to the hexagonal H_{II} phase. It is concluded that these model systems provide an exciting and valuable tool in revealing fundamental aspects of membrane structure and function.

An essential feature during the process of membrane fusion is destabilization of the bilayer packing. In Chapter 1, a brief review is given of the factors which determine the self-organization of amphiphiles into a bilayer. The self-assembly process is favored by both hydrophobic interactions and the large difference in cohesive energy density between water and hydrocarbons. These attractive interactions are opposed by repulsive head group interactions. Thus, a delicate balance of attractive and repulsive interactions dictates the self-assembly process. The concept of the molecular packing parameter provides a correlation between the molecular structure and the type of aggregate formed. It appears that amphiphiles must possess a cylindrical shape in order to be able to form bilayers. The fusion process of phospholipid vesicles and the frequently observed, spontaneous instability of vesicles prepared from synthetic amphiphiles is briefly discussed.

In Chapter 2 it is unequivocally demonstrated, for the first time, that vesicles prepared from synthetic amphiphiles are able to fuse. Fusion of didodecyldimethylammonium bromide (DDAB) vesicles can be induced by the dianion of dipicolinic acid (DPA^{2-}). The fusion process was monitored by employing electron microscopy and a resonance energy transfer (RET) fusion assay. In addition, turbidity and fluorescence polarization measurements revealed that fusion is conferred to the vesicles in the aggregated state, and that the bilayers should be in a fluid or liquid-crystalline phase. It is shown that binding of DPA^{2-} leads to a (partial) dehydration of the DDAB head groups and, concomitantly, to a reduction of the head group mobility. These alterations, which are already completed below the threshold DPA^{2-} concentration for the induction of aggregation (*i.e.* the alterations occur on individual vesicles) are discussed in terms of the formation of so-called "*cis*" complexes. These



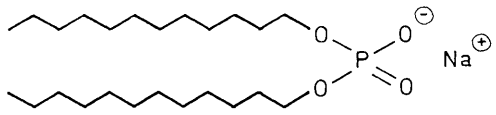
DDAB



DPA^{2-}

"*cis*" complexes are complexes that consist of the fusogenic agent and two adjacent head groups within the lateral plane of the bilayer. Although fusion requires a higher concentration of DPA^{2-}, no further alterations in the bilayer and the bilayer-water interface could be detected. It is assumed, therefore, that fusion is induced by a local perturbation of the bilayer. Presumably, this perturbation involves the formation of a "*trans*" complex between the fusogenic agent and the head groups of two closely apposed bilayers.

In Chapter 3 the Ca^{2+}-induced fusion of didodecylphosphate (DDP) vesicles is described. The change in head group structure (dimethylammonium *versus* phosphate) has a pronounced effect on the structural and fusogenic properties of the vesicles. Differences in the bilayer packing and the fusion activity between DDP vesicles and DDAB vesicles are attributed to alterations in charge (negatively *versus* positively charged, respectively), size, and hydration (the head group of DDP is smaller and more strongly hydrated than that of DDAB). Interestingly, in the case of DDP vesicles, the fusogenic agent (Ca^{2+}) is able to induce a polymorphic transformation from a bilayer to a hexagonal H_{II} packing of the DDP molecules. This is a consequence of the smaller size of the head group of DDP relative to that of DDAB. A detailed analysis of this bilayer-to-hexagonal H_{II} phase transition using electron microscopic techniques such as selected area electron diffraction and cryo electron microscopy, and ^{31}P-NMR spectroscopy, led to a proposal for the involvement of an unstable inverted micellar structure (IMI), as an intermediate in both the fusion process and the bilayer-to-hexagonal H_{II} phase transition of the DDP vesicles. The formation of an IMI is triggered by a (partial) dehydration of the DDP head groups and the subsequent formation of a "*trans*" complex between two closely apposed bilayers. The observation that only large fused vesicles transform into hexagonal H_{II} tubes indicates that the number of IMI's at the contact-site between both bilayers



DDP

determines whether fusion or the bilayer-to-hexagonal H_{II} phase transition occurs.

To obtain a better insight into the molecular organization of the DDP bilayer and into the structural requirements for the formation of an IMI, the DDP head group conformation and the dynamical behavior of DDP in the bilayer was studied. Using ^{31}P-NMR and fluorescence polarization techniques and an order parameter approach (Chapter 4) it is shown that the molecular long axis bisects both the (R)-O-P-O-(R) and O-P-O angle while the motion of the DDP molecule can be described by a wobble of the long axis within a cone which makes an angle of *ca.* 55^{o} with the bilayer normal. The head group conformation is related to the ability of the head group *per se*, to participate (in the presence of the fusion-inducing cation) in the formation of the "*cis*" and "*trans*" complex.

In Chapter 5 it is shown that a change in pH strongly affects the physical state of the DDP bilayer. This in turn has a large influence on the propensity of the vesicles to undergo fusion. The bilayer fluidity and the fusion activity appear to be minimal at the effective pK_a of the DDP head group. Interestingly, the head group region of the bilayer maintains a reduced mobility below the effective pK_a, whereas the mobility of the interior of the bilayer increases again. The experimental results suggest that at low pH the DDP vesicles possess an organization that is intermediate between a lamellar and a hexagonal H_{II} phase. However, Ca^{2+} is still necessary for the induction of fusion and the transformation of the DDP molecules into a hexagonal H_{II} arrangement.

One of the key events in the fusion process is dehydration of the head groups, which is necessary for lowering the strong repulsive hydration forces which would otherwise keep the membranes separate. Therefore, the influence of a dehydrating agent, poly(ethylene glycol) (PEG),

$$\left[CH_2CH_2O \right]_m$$

PEG

was investigated. This work also bears relevance to a better understanding of polymer-amphiphile interactions. In Chapter 6 it is shown that, indeed, the addition of PEG with a molecular weight of 8,000 facilitates fusion. However, PEG with a molecular weight of 20,000 inhibits membrane

fusion. This peculiar effect of the molecular weight is attributed to a difference in clouding temperature. It is suggested that PEG with a molecular weight of 20,000 will undergo a microscopic phase separation at the bilayer-water interface because at the experimental conditions PEG-PEG interactions and presumably DDP-PEG interactions are favored over PEG-water interactions. As a result, the high molecular weight polymer interacts strongly with the DDP bilayer. This, in turn, results in "steric" stabilization of the vesicles, thereby inhibiting membrane fusion.